## REMARKS

### I. Status of the Claims

Claims 1-9 and 11 are pending and stand rejected under 35 U.S.C. §103. The specific grounds for rejection, and applicants' response thereto, are set out in detail below.

Applicants have provided a substantially revised claim set that almost exclusively provides clarifying amendments to the existing claims. As such, no new matter is introduced by the amendments and new claims.

### II. Rejections Under 35 U.S.C. §103

#### A. Barquinero and Muzya

Claims 1-8 stand rejected as obvious over Barquinero *et al.* in view of Muzya *et al.* Barquinero is cited as teaching an ELISA assay to assess anti-PAF levels in autoimmune diseases and syphilis, and Muzya is cited as teaching that antibodies binding to phosphocholine also bind to PAF, lyso-PAF and acyl analogs of PAF, and are found in women with gynecologic disorders, including spontaneous abortion. Applicants traverse.

##### *1. Interview*

Applicants wish to thank the examiner and her supervisor for the courtesy of an in-person interview, held at the Patent and Trademark Office, on November 15, 2006, including Examiner Cook, SPE Long Le, and the undersigned. During the interview, the undersigned addressed the references cited above, as well as Baldo *et al.* (discussed below) and explained the limitations that one must place on the interpretation of those references. While agreement was not reached, the substance of the interview was believed helpful in advancing the prosecution. It was agreed that applicants would reiterate their position with regard to all of the references and non-

obviousness in a formal response to the action, likely accompanied by a Rule 132 declaration discussing the Muzya reference. ***As urged at the conclusion of the interview, applicants request that should the examiner fail to find the claims as submitted herein allowable, a telephone call to the undersigned be initiated prior to issuance of a further action.***

### *2. Lack of a Prima Facie Case*

First, applicants would like to draw the examiner's attention to new claim 1, which now reads as follows:

> A method for diagnosing risk of spontaneous abortion comprising: (a) contacting a sample of body fluid with an antigen capable of binding to an antibody to platelet activating factor (PAF), (b) assessing the presence and/or concentration of said antibodies in the sample by measuring antibodies bound to said antigen; and (c) diagnosing the risk of spontaneous abortion based on the presence and/or concentration of said antibodies in said sample.

Thus, the claim as written is quite straightforward – an assay for antibodies that bind to PAF as a diagnostic for spontaneous abortion.

As discussed above, the examiner has cited Barquinero as teaching a ELISA for anti-PAF antibodies in the context of autoimmune diseases and syphilis.[1] The examiner then turns to Muzya in an attempt to correlate anti-PAF antibodies with spontaneous abortion.[2] However, it is applicants' contention that one cannot draw simple conclusions from Muzya regarding the significance of anti-PAF antibody detection reported therein. As explained in the accompanying declaration of Dr. Frostegård, Muzya apparently pre-selected serum containing

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[1] As applicants' representative pointed out during the interview, however, Barquinero does ***not*** teach a correlation with anti-PAF antibodies and autoimmune disease, and the examiner agreed.
[2] Applicants are providing a certified translation of the entire Muzya *et al.* paper, as the examiner had (and apparently has) only access to a translated abstract.

phosphatidylcholine antibodies. Only then were these pre-selected sera screened for cross-reactivity with PAF and various PAF analogs.

Thus, because of the limitations one must use in interpreting the data of Muzya, applicants submit one cannot readily conclude that anti-PAF antibodies were, in fact, correlated with spontaneous abortion. As such, the cited art lack sufficient motivation to examine anti-PAF for diagnosis of this condition. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

**B. Barquinero, Muzya and Baldo**

Claims 9 and 11 stand rejected as obvious over Barquinero *et al.*, in view of Muzya *et al.* and Baldo *et al.* The first two references are cited as above, whereas Baldo is cited for phospholipid analogs that are sufficient to induce anti-PAF antibodies, PAF being known to be insufficiently antigenic to produce anti-PAF antibodies in standard immunization schemes. Once again, applicants traverse.

Admittedly, Baldo provides only analogs of PAF. While one interested in ***generating*** anti-PAF antibodies might be motivated to use various of Baldo's phospholipids due to the apparent low antigenicity of PAF, one interested in ***identifying*** anti-PAF antibodies would have ***no*** reason to use something other than ***PAF itself***. PAF is not in short supply, expensive, or difficult to use. Moreover, as noted by Barquinero, there is a difference in the ability of charged phospholipids to ***cross-react*** with anti-PAF, and in their ability to ***compete*** with PAF for anti-PAF binding. Indeed, Muzya also comments that "[i]n contrast to highly specific antibodies to PAF, aPC antibodies are not highly specific and reactive with other phospholipids." Muzya, 2nd para. following Table 2. This is a clear condemnation of using anything other than PAF in anti-PAF assays.

Thus, applicants submit there is no motivation for using things other than PAF in an assay for anti-PAF – indeed, there is instruction against doing so. For this additional reason, the rejection is justified. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

## IV. Conclusion

In light of the foregoing, applicants respectfully submit that all claims are in condition for allowance, and an early notification to that effect is earnestly solicited. Should there be any questions regarding this submission a telephone call to the undersigned attorney at (512) 536-3184 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

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The Patent Translation Company

# CERTIFIED TRANSLATION

In the matter of filing of a translation of a Russian journal article by a collective of authors, namely G.I. Muzya, I.V. Ponomareva, V.I. Kulikov, G.T. Sukhikh, entitled

ВЗАИМОДЕЙСТВИЕ АНТИФОСФАТИДИЛХОЛИНОВЫХ АНТИТЕЛ С ФОСФОЛИПИДНЫМ ФАКТОРОМ АКТИВАЦИИ ТРОМБОЦИТОВ И ЕГО СТРУКТУРНЫМИ КЛЕТОЧНЫМИ АНАЛОГАМИ

"Reaction of antiphosphatidylcholine antibodies with thrombocyte-activating phospholipid factor and its structural cellular analogues",

**I, Eyvor Fogarty**

**Translator to** **The Patent Translation Company Ltd**

**do solemnly and sincerely declare as follows:**

1. I am fully conversant with the Russian and English languages.

2. The attached translation, which I have made in English of the aforementioned Russian journal article, is certified to be, to the best of my knowledge and belief, a true and correct translation.

**Signature** .....Eyvor Fogarty.....

**Name: Eyvor Fogarty**

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UDC 618.3-092:812.087.1]-078-33

# Reaction of antiphosphatidylcholine antibodies with thrombocyte-activating phospholipid factor and its structural cellular analogues

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The high proportion of antiphospholipid antibodies to membrane phospholipids is often associated with obstetrical pathology; this includes recurrent foetal loss, intrauterine growth retardation, hypertension in pregnancy, preeclampsia and thromboembolic complications [15]. In such cases, the blood serum of patients contains antibodies to the main cellular phospholipids such as cardiolipin, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, sphyngomyelin and phosphatidylcholine [10]. Study of the specific mechanisms of antiphospholipid antibody participation in the development of the pathology of pregnancy continues [14, 15]. It has been suggested that the rise observed in pregnancy in the level of phospholipids in the blood due to an increase in their anabolism, the deportation of syncytiotrophoblast microvilli and the release of phospholipid vesicles by the placenta stimulate the production of antiphospholipid antibodies [10].

Antiphospholipid antibodies (aPL) are apparently reactive not only with 'excess' phospholipids in the blood serum but also with lipoproteins and cells containing phospholipid antigen determinants on the cell surface [14].

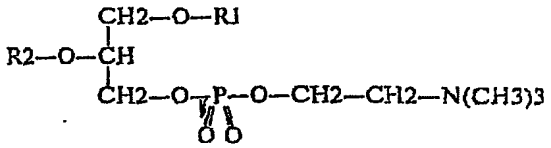
It is known that given the correct stimulus virtually all cells in mammals will release a universal phospholipid bioregulator, platelet-activating factor (PAF) [3] and its choline-containing cellular analogues - acyl and plasmalogen [4]. Phospholipid PAF is involved in the regulation of the blood clotting system, the cardio-vascular system and the immune system and is a mediator of inflammation with a range of etiologies, of allergic reactions and many other pathophysiological processes. PAF plays a major role in mammalian reproduction, with an effect on virtually all stages of the reproductive process, from functional development of the gametes, fertilisation and

embryo implantation to childbirth [6]. Since PAF is in terms of its chemical structure a choline-containing phospholipid, it may be expected that aPL antibodies and aPC antibodies in particular would be reactive with PAF and its structural analogues, with an effect on their biological activity.

The aim of this study was to investigate the reaction of blood serum containing antiphosphatidylcholine antibodies with PAF and its structural analogues.

*Research method*

Preparations of highly purified phospholipids of the following structure were used in the research project.




1) phosphatidylcholine: R1 is C16:0 and C18:0 fatty acid residues; R2 is C18:1 and C18:2 fatty acid residues
2) lysophosphatidylcholine: R1 is C16:0 and C18.0 fatty acid residues; R2 is H
3) phospholipid PAF: R1 is (CH2)15,17CH3; R2 is CH3CO;
4) PAF lysoderivative (lyso-PAF): R1 is (CH2)15, 17CH3; R2 is H
5) acyl analogue of PAF (1-acyl-PAF): R1 is C16:0 and C18.0 fatty acid residues; R2 is CH3CO.

Phosphatidylcholine is separated from egg yolks by the usual method [1]. Lysophosphatidylcholine (1-acyllysoglycero-3-phosphocholine) was obtained by cleaving egg phosphatidylcholine with phospholipase A2 and purified by column chromatography in L 100/160 μm silica gel [1]. 1-0-alkyllyso-*sn*-glycero-3-phosphocholine (lyso-PAF) was obtained by hydrogenating bovine heart choline plasmalogens, followed by alkaline hydrolysis as described earlier [1]. Phospholipid PAF was obtained by acetylating 1-0-alkyllyso-sn-glycero-3-phosphocholine with acetic anhydride in a chloroform medium and purifying by column chromatography in L 100/160 μm silica gel [2]. The PAF acyl analogue (1-acyl-2-acetyl-*sn*-glycero-3-phosphocholine) was obtained by acetylating the lysophophatidylcholine with acetic anhydride in a chloroform medium and purifying by column chromatography in L 100/160 μm silica gel [7].

Murine monoclonal antibodies to human immunoglobulins (IgM, IgG), labelled horseradish peroxidase (Institute of Virus Preparations, Moscow), gelatine (N.A. Semashko Moskhimpharmpreparat), o-phenylendiamine (Sigma), hydrogen peroxide (Reakhim) and polystyrene microplates manufactured by GosNIIMedpolimer (Moscow) were used for the enzyme immunoassay (EIA). Blood serum samples taken in the Scientific Centre of Obstetrics, Gynaecology and perinatology of the Russian Academy of Medical Sciences from patients with recurrent foetal loss, late toxicosis in pregnancy, history of perinatal foetal death, infertility and unsuccessful attempts at *in vitro* fertilisation and embryo transfer.

EIA was used to study the manner in which aPL antibodies bind with PAF and its structural analogues. Highly purified phospholipids (phosphatidylcholine, PAF, lyso-PAF, 1-acyl-PAF, lysophosphatidylcholine) were dissolved in a 50 μg/ml methanol concentration. The resultant phospholipid solutions were placed onto polystyrene microplates in quantities of 50 μm per well and incubated at 37°C for 18 ± 2 hrs. After each stage of the assay the plates were washed 4 times with 0.01 M phosphate buffer solution (pH 7.4 ± 0.2). After adsorption of the phospholipids the wells were treated with a 0.5% gelatine solution, 100 μm per well, at 20 ± 2 °C for 1.5 hr. A phosphate buffer solution containing 0.5% gelatine was used for cultivating the test samples of blood serum and conjugates. 75 μl assay samples of blood serum cultivated in a 1:50 proportion were inserted per well and incubated at 20 ± 2 °C in an agitator for 1.5 hr. Conjugates of murine monoclonal antibodies, with horseradish peroxidase, to human IgM and IgG, in 1:100000 and 1:50000 proportions respectively, were placed in the wells in amounts of 50 μl per well and incubated at 20 ± 2 °C in an agitator for 1 hr. After washing, a chromogen substrate solution containing o-phenylendiamine and hydrogen peroxide was added to the wells and the optical density (OD) was measured after 10 minutes at 492 nm using a Labsystems Multiscan MCC/340 photometer. The results of the assay were considered positive if the average OD of the assay sample was greater than the total of the average OD for the negative controls and two average mean square deviations.

*Results and Discussion*

To study the way antiphosphatidylcholine (aPC) antibodies bind with phospholipid PAF and its structural analogues, blood serum containing IgM, or IgM and IgG phosphatidylcholine antibodies was taken from patients presenting with obstetric and gynaecological pathologies. In

the case of the patient with late toxicity in pregnancy the IgG level was relatively higher than the IgM level, while in the other cases the IgM level was higher.

The EIA results indicated that serums containing IgM and IgG aPC antibodies react *in vitro* with the PAF and its analogues adsorbed onto the polystyrene plates. In addition, the linking of IgM antibodies with phosphatidylcholine was approximately 1.5 - 2 times higher than with PAF, lyso-PAF and 1-acyl-PAF adsorbed under the same conditions, and 3 times higher than with lysophosphatidylcholine (Table 1). No substantial differences were found in the degree of the reaction of aPC antibodies with PAF, lysoPAF and 1-acyl-PAF. The cross reaction typical for antiphospholipid antibodies had obviously occurred in this case.

*Table 1*

Level of IgM aPC antibodies in blood serum of patients with obstetric and gynaecological pathologies, and the binding with PAF and its structural analogues found from EIA

| Patient group | Phospholipid tested | | | | |
|---|---|---|---|---|---|
| | Phosphatidyl-choline | PAF | Lyso-PAF | 1-acyl-PAF | Lyso-phosphatidyl-choline |
| Patients with death of infant in neonatal period | 0.580 ± 0.035 | 0.759 ± 0.044 | 0.387 ± 0.023 | 0.386 ± 0.022 | 0.268 ± 0.015 |
| Patients with foetal loss | 0.320 ± 0.016 | 0.576 ± 0.034 | 0.243 ± 0.014 | 0.208 ± 0.012 | 0.145 ± 0.018 |
| Patients with late toxicosis in pregnancy | 0.400 ± 0.024 | 0.645 ± 0.065 | 0.293 ± 0.017 | 0.229 ± 0.013 | 0.378 ± 0.022 |
| Patients with infertility | 0.541 ± 0.031 | 0.727 ± 0.073 | 0.410 ± 0.024 | 0.356 ± 0.021 | 0.268 ± 0.016 |
| Healthy fertile women | 0.050 ± 0.003 | 0.126 ± 0.007 | 0.062 ± 0.004 | 0.051 ± 0.003 | 0.074 ± 0.004 |

Note: In Tables 1 and 2 the values given are for average OD at 492 nm ± σ

In the serum of patients with low levels of IgG aPC antibodies the differences in the way they bind with PAF and its analogues were slight (Table 2). However in the serum of the patient with late toxicosis in pregnancy a high level of IgG antibodies reactive with PAF and, significantly, to a lesser extent with its analogues was noted. It is not impossible that this patient had specific antibodies to PAF.

*Table 2*

Level of IgG aPC antibodies in blood serum of patients with obstetric and gynaecological pathologies, and the binding with PAF and its structural analogues found from EIA

| Patient group | Phospholipid tested | | | | |
|---|---|---|---|---|---|
| | Phosphatidyl-choline | PAF | Lyso-PAF | 1-acyl-PAF | Lyso-phosphatidyl-choline |
| Patients with death of infant in neonatal period | 0.189 ± 0.011 | 0.227 ± 0.013 | 0.141 ± 0.015 | 0.098 ± 0.006 | 0.086 ± 0.005 |
| Patients with foetal loss | 0.126 ± 0.008 | 0.250 ± 0.016 | 0.127 ± 0.009 | 0.089 ± 0.006 | 0.076 ± 0.008 |
| Patients with late toxicosis in pregnancy | 0.574 ± 0.034 | 1.011 ± 0.059 | 0.152 ± 0.010 | 0.097 ± 0.006 | 0.149 ± 0.009 |
| Patients with infertility | 0.085 ± 0.005 | 0.221 ± 0.014 | 0.114 ± 0.007 | 0.089 ± 0.006 | 0.050 ± 0.003 |
| Healthy fertile women | 0.061 ± 0.004 | 0.134 ± 0.008 | 0.064 ± 0.004 | 0.057 ± 0.004 | 0.050 ± 0.003 |

It is known that the antibodies to PAF may be evoked in rabbits after the introduction of PAF preparations containing C6:0 and C:12 alkyl residues, and PAF analogues (1-0-(ω-oxyalkyl)-2-acetyl-*sn*-glycero-3-phosphocholine, 1-0-(15'-carboxypentadecyl)-2-N, N-dimethylcarbamoyl-*sn*-glycero-3-phosphocholine), covalently linked to methylated BSA [8, 11, 17, 18]. The identified antibodies to PAF were highly specific and were not reactive with lyso-PAF, PAF enantiomer, PAF methoxy analogue, lysophosphatidylcholine, phosphatidylcholine or PAF analogues containing propionic or butyric acid residues at the *sn*-2 position [8, 11, 17]. With the different molecular types of PAF containing C16:0, C18:0 and C18:1 alkyl residues at the *sn*-1 position, there were some small variations in the bonding of the antibodies, and the greatest bonding of antibodies was observed in C18:1 PAF [8]. These results indicate that the high specificity of antibodies to PAF depends on the recognition of the acetyl group at the *sn*-2 position and the trimethylammonium group of phosphocholine in the PAF molecule [17].

In contrast to the highly specific antibodies to PAF, aPC antibodies are not highly specific and are reactive with other phospholipids. It has been shown that antibodies to phosphatidcholine can be evoked in experimental animals by introducing erythrocytes, an emulsion of dipalmitoyl phosphatidylcholine in BSA or phosphatodylcholine liposomes, and they can also be produced by hybridoma technology [9, 12, 13, 16, 19]. aPC antibodies are also capable of binding with

lyso-phosphatidylcholine and sphyngomyelin [12], that is, they are capable of recognition of phosphocholine fragments of the polar part of phospholipids.

The results of this study show that IgM and IgG aPC antibodies in blood serum from patients with obstetric and gynaecological pathologies are capable of binding *in vitro* with PAF and its structural analogues which differ from PAF in the type of bond at the *sn*-1 position: a simple ether bond in the case of PAF and an ester bond in the case of 1-acyl-PAF.

What are implications of this observable reaction of aPC antibodies with PAF and its analogues in the pathogenesis of antiphospholipid syndrome (APS)? Thrombosis of the vessels of the placenta is thought to be the main mechanism in the development of obstetric pathology, with one of the causes of its occurrence being the major role played by the reaction of aPL antibodies with endothelial cells and thrombocytes [14]. It has been shown that the binding of aPL antibodies with endothelial cells leads to a reduction in the synthesis of prostacyclin, while their reaction with thrombocytes initiates the activation of thrombocytes and subsequent increase in the synthesis of thromboxane A2 and the release of adenosine diphosphate (ADP) [15]. At the same time, due to the presence of anticardiolipin antibodies, the endothelial cells release PAF [6]. Thus the increased production by cells of proaggregating agents such as PAF, thromboxane A2 and ADP along with the reduction in the synthesis of prostacyclin can cause the formation of intravascular aggregates of thrombocytes.

It is known that, in the blood circulation, PAF, released by cells binds with albumin and plasma lipoproteins [2] while free PAF is cleaved by acetylhydrolase associated with low density lipoproteins [5]. aPC antibodies can, apparently, bind with PAF in the microenvironment of cells actively producing PAF. It can be suggested that the formation of a compound with an antibody can inhibit the cleavage of PAF by acetylhydrolase.

An another important implication of the reaction of aPC antibodies with PAF may be the disturbance of the process of fertilisation of oocytes by spermatozoids. It is known that PAF stimulates spermatozoid motility, the acrosome reaction, and the process of fertilisation and implantation of the embryo [6]. Apparently aPC antibodies can significantly disturb these processes by removing PAF from the interaction of cells in the reproductive system. It is possible that unsuccessful attempts at *in vitro* fertilisation may be associated with a disturbance of the process of fertilisation and implantation of the embryo as a result of the binding of PAF

with aPC antibodies. It is therefore possible to suggest new links between APS and disturbances of the fertilisation processes in humans.

**CONCLUSIONS**

1. Antiphosphatidylcholine antibodies in the blood serum of patients with an obstetric and gynaecological pathology bind *in vitro* with phospholipid PAF, PAF lysine derivatives and PAF acyl analogues.

2. Antiphosphatidylcholine antibodies bound with PAF and its structural cell analogues are likely to be associated with the presence of phosphocholine fragments in the structure of certain phosphoglycerides.

LITERATURE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Trans notes:
1. *thrombocyte activating phospholipid factor*: The literal translation has been used in the title, since it is a title. Elsewhere in the text the more usual English platelet activating factor, PAF, has been used.

2. *structural cellular analogues*: this Russian term has been shortened to 'structural analogues' throughout the translation.

3. *Patient*: in this text, the Russian uses the word 'female patient'.

4. *Phosphatidylinositol*: This term has been used to translate the Russian 'phosphatidylinosite'.

5. *late toxicosis in pregnancy*: the Russian term, 'OPG-gestoz', was introduced in 1987 for late toxicosis in pregnant women; the *O G P* stands for oedema, proteinuria and hypertension.